The Effect of Lairage on Salmonella Isolation from Market Swine^{†‡}

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ABSTRACT

The objective of this paper was to evaluate the effect of lairage (holding >12 h during transport to slaughter) in clean facilities on Salmonella isolation from market swine. We tested 30 market-bound pigs (about 240 lb [110 kg]) on each of 10 occasions from an Iowa farrow-to-finish operation with about 600 sows. All pigs were slaughtered, and samples were collected at a large Midwest abattoir. On the farm, fecal samples were collected for culture of Salmonella. Pigs were alternately assigned to a lairage treatment (holding in a clean, disinfected facility at the National Animal Disease Center) group or a control group (remaining on the farm). After about 18 h, both groups were transported (about 137 km) to a large Midwest abattoir, commingled, and slaughtered. After slaughter, samples were collected for culture of Salmonella (feces from the distal colon, ileocecal lymph nodes, cecal contents, ventral thoracic lymph nodes, subiliac lymph nodes, and carcass swabs). Diaphragm sections were collected for serum ELISA. Salmonella enterica Derby was the only serotype isolated from farm fecal samples (3.4%, 10 of 290). Multiple serotypes (n = 17) were isolated from 71.8% (196 of 273) of the pigs when abattoir-collected samples were cultured: cecal contents (21.2%, 58 of 273), distal colon contents (52%, 142 of 273), and ileocecal lymph nodes (43.6%, 119 of 273). There were lower Salmonella isolation rates from the lairaged pigs (P < 0.05). The predominant serotype isolated at the abattoir varied by week of the study. This study suggests that pigs became internally contaminated with Salmonella after leaving the farm, possibly while in the abattoir holding pens, and that 18 h lairage, in clean facilities, does not increase shedding.

Salmonella and other foodborne human pathogens are becoming an increasing concern for the pork industry. In the United States, these pathogens are being considered another measure of overall pork quality (8). The European Union is now requiring all member states to initiate monitoring programs for Salmonella in pigs (15). Along the farm to fork continuum, there are many processes that may affect prevalence of these pathogens and increase their risk to human health. These include: on-farm production, transport, holding (lairage) at the abattoir or other collection points, the slaughter process, and fabrication, as well as retail and consumer handling. Measuring the impact each process has on Salmonella prevalence is a critical first step in developing pathogen reduction strategies.

Higher Salmonella prevalence rates have been reported from pigs tested after shipment to slaughter, compared to when they were tested on-farm (3, 12, 16, 17, 27, 30, 31). Based on these studies, three general reasons for an increase in Salmonella isolation rates can be deduced: transport stress, lairage stress with commingling, and differences in

the samples analyzed (pre- and posttransport). Transport stress might cause a latent carrier to shed the organism (28, 31). Stress might also increase a noninfected animal's susceptibility to infection (1, 2, 10, 28). Except in one study where pigs started with very high shedding rates, increased lairage time has been shown to increase Salmonella shedding (3, 5, 20, 21). Lairage might cause the same stress effects as transport. Additionally, transport and lairage usually include fasting, which may increase shedding (12-14, 31). Lairage may also allow commingling with infected animals from other farms. Notably, most previous lairage studies occurred at slaughter facilities with likely high levels of environmental contamination (5, 16, 21, 29). In these transport and lairage studies, it is difficult to parse out the effect of lairage from transport, as all lairaged animals were transported, and most transported animals were lairaged (>12 h) before slaughter and sample collection. The third reason for pre- and posttransport Salmonella prevalence discrepancies might be sample collection differences. These differences include: volume of sample, sample source, and number of animals tested before and after transport or lairage (7, 17). Before shipment, the only antemortem samples readily available for culture are feces. After shipment and slaughter, various samples are available.

The best protocol to assess *Salmonella* prevalence is not known. The research noted above examined gut contents (cecal or rectal) or gut-associated lymph nodes (ileocecal, mesenteric, or portal) to identify *Salmonella* contamination. However, modern slaughter practices make every

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attempt to prevent these *Salmonella* sources from contaminating carcasses. Currently, the Federal Safety and Inspection Service uses carcass surface contamination to measure the effectiveness of the plant's Hazard Analysis and Critical Control Point program. However, surface contamination likely reflects the quality of in-plant practices and may not be a useful measure of preharvest prevalence. Additionally, the correlation of carcass contamination with human risk has not been established. Tissues such as the superficial inguinal, mandibular, and thoracic lymph nodes may be a better measure of food safety risk, as they may be included in retail pork (25). In the Danish *Salmonella* control program, preharvest *Salmonella* prevalence is monitored with a serum ELISA (22).

The objectives of this study were (i) to evaluate the effect of lairage (holding >8 to 12 h during transport to slaughter) in clean facilities on *Salmonella* isolation from market swine, and (ii) to compare prevalence rates from gut-associated tissues, systemic lymph nodes, carcass swabs, and serum ELISA.

MATERIALS AND METHODS

On-farm sampling. This study was conducted in cooperation with a privately owned farrow-to-finish operation (about 600 sows) in the Midwest. Study animals were housed in three separate buildings on two premises. For 10 weeks between May and August, 30 pigs (about 110 kg) were randomly selected from the approximately 200 destined for market that week. No more than five pigs were selected from each pen. Pigs were identified with a unique slap tattoo on both rear hams. Feces (1 g) were collected using a fecal loop (Jorgensen, Loveland, Colo.). Feces were placed immediately into enrichment media (Difco Laboratories, Sparks, Md.). Fecal samples were cultured for the presence of Salmonella spp. as previously described (32). Preenrichment included separate tubes of GN-Hajna broth (Difco) (24 h at 37°C) and tetrathionate broth (Difco) (48 h at 37°C) followed by enrichment in Rappaport-Vassiliadis media (Difco) (24 h at 37°C). A portion of the Rappaport-Vassiliadis media was then streaked to brilliant green sulfa agar (Difco) (24 h 37°C) and XLT4 agar (Difco) (24 h at 37°C), after which suspect colonies were picked and transferred to triple sugar iron and lysine iron agar slants (24 h at 37°C). Biochemically suspect isolates were serogrouped with *Bac*to Salmonella O antiserum (Difco). Positives were serotyped at the USDA, National Veterinary Services Laboratories (Ames, Iowa). Blood was collected from the anterior vena cava by venipuncture. Blood was allowed to clot for 12 h at 4°C and centrifuged. Serum was divided into 1-ml aliquots, frozen, and stored at -70° C.

Lairage treatment. At the time of sample collection, every other pig was designated for inclusion in the lairage treatment group. After all 30 head were tested, the 15 to be lairaged were transported (about 65 km) in a clean, disinfected trailer to a facility at the USDA, ARS, National Animal Disease Center. The facility was fully enclosed, with solid concrete floors that were washed and disinfected after each use. Lairaged pigs were provided only water. The remaining pigs (controls) were left in their original pens for shipment to the abattoir the following morning. They continued to receive normal feed and water. Beginning in week 7 of the study, we also began taking 1-g fecal samples from lairaged pigs after about 18 h lairage, as pigs were being loaded. The pigs were shipped in the trailer used the previous day about

70 km to a high capacity (>10,000 head/day) abattoir. At the same time, the controls were transported about 135 km, in a clean, disinfected trailer, to the same abattoir. Both groups of pigs were shipped approximately the same total distance, as it is only a 3-km detour to the National Animal Disease Center lairage facility. Lairaged pigs and controls were commingled and held for 2 to 3 h at the abattoir before slaughter.

Abattoir samples collected. The pigs were slaughtered in the plant's routine manner. Carcasses were serially identified with an edible ink number. During evisceration, the lungs were serially numbered to match the carcass numbers. Samples were collected from viscera and carcasses at separate locations within the plant. The carcasses were rinsed in acetic acid but were not quick frozen. In the cooler, a sponge swab (Fisher Scientific, Springfield, N.J.) soaked in buffered peptone water was rubbed on three separate 100-cm² sites on the gluteal muscles, abdomen, and jowl of the carcass. Visceral samples collected included about 1 g of lymph nodes located near the ileocecal junction (ILC), 10 g of cecum contents (CC), and 10 g of distal colon contents (DCC). When sufficient contents were not available, a 6- to 8-in. section of the distal colon was removed, longitudinally incised, and placed in enrichment media.

Samples were transported, on ice, to the National Animal Disease Center and were processed within 2 h of collection. Tissue samples were mixed with 5 ml of buffered peptone water and macerated with a rubber mallet. Each sample was then homogenized using a stomacher at 260 rpm for 1 min. One milliliter of supernatant was then added directly to each preenrichment medium (10 ml) and cultured and serotyped as previously described. Cecal and colon contents (10 g) were added directly to enrichment media. All samples were cultured for Salmonella as previously described. Diaphragm samples were frozen (-20°C) and sent, along with farm-collected serum, to the laboratory of Dr. D. L. Harris, Iowa State University, where the Danish ELISA was performed (24). The level of antibodies was measured in the ELISA by a colorimetric response expressed as optical density percentage (OD%). In this study, a pig was considered ELISA positive if either the blood serum or diaphragmatic meat juice was greater than or equal to the cutoffs of OD% \geq 20 or 40. Results were analyzed for both cutoff values.

Analysis. A 95% confidence interval was calculated for the proportion of positive samples (19). Comparison of the intervals allows determination of a difference in the prevalence at P < 0.05. Salmonella isolation rates for lairaged and control pigs were compared by chi-square tests. A variable, any abattoir, was calculated to be positive if an animal was culture positive for any of the six samples collected at the abattoir. Serotype diversity among samples was compared by means of a simple diversity index (equation 1). The number of different serotypes, in equation 1, also included those reported as multiple serotypes from a single sample. Additionally, the weekly distribution of predominant serotypes (more than or equal to four isolations) was evaluated.

Diversity index = (number of serotypes
$$> 1$$
) (1)
 \div number of typable isolates

RESULTS

Table 1 summarizes the number and percentage of pigs (upper and lower 95% confidence limits) that were *Salmonella* positive for each of the samples tested. Of the 290 pigs with complete ante- and postmortem data, only 3.4% (10 of 290) were positive by on-farm fecal culture. Using a combination of all abattoir-collected tissues tested, 71.8%

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TABLE 1. Number and percentage of market swine positive for Salmonella spp. by sample collected

Sample source	No. of pigs positive	No. of pigs tested	% of positive pigs	Lower 95% confidence limit	Upper 95% confidence limit 5.6 26.1		
On-farm fecal (1 g)	10	290	3.4	1.3			
CC (10 g)	58	273	21.2	16.4			
DCC (10 g)	142	273	52.0	46.1	58.0		
Ileocecal lymph nodes	119	273	43.6	37.7	49.5		
Subiliac lymph nodes	1	272	0.4	0.0	1.1		
Ventral thoracic lymph nodes	1	272	0.4	0.0	1.1 1.1		
Carcass swabs	1	272	0.4	0.0			
Any abattoir ^a	196	273	71.8	66.4	77.1		

^a Any abattoir = positive on any sample except on-farm fecal.

(196 of 273) of these pigs were *Salmonella* positive. Only 28% (77) pigs were *Salmonella* negative on all abattoir-collected samples. Two of these negative pigs tested positive on-farm. *Salmonella* spp. were isolated from 21.2% of cecal contents (58 of 273), 52% of distal colon samples (142 of 273), and 43.6% of ileocecal lymph node homogenates (119 of 273). The DCC had the highest isolation rate, which was significantly higher (P < 0.05) than the CC but not higher than the ILC. *Salmonella* spp. were recovered from one carcass swab, one ventral thoracic lymph node, and one subiliac lymph node, each from a different pig. All pigs positive with these samples were also positive for at least one visceral sample (CC, DCC, or ILC).

A comparison of isolation rates for lairaged and control animals showed lower isolation rates in the lairaged pigs when comparing ILC (35.6 versus 50.7%), DCC (44.4 versus 59.4%), and any abattoir-positive (65.2 versus 78.3%) samples (P < 0.05) (Fig. 1).

During the study, we noticed the increase in isolation rates for abattoir-collected samples compared to farm fecals. Therefore, for weeks 7 to 10, we collected an additional 1 g of fecal sample from lairaged pigs after about 18 h lairage. For the 50 lairaged pigs with complete results, only one was positive on-farm (week 7). After about 18 h lairage, a different pig was positive by fecal culture (week 7). However, at slaughter (4 to 5 h later), 70% (35 of 50) of these pigs had at least one positive tissue sample.

Based on the serum ELISA, only 1.7% (5 of 293) of pigs showed evidence of historical *Salmonella* infection at the OD% \geq 40 cutoff. At an OD% \geq 20, 6.9% (20 of 291) were positive. Of the 10 pigs that were culture positive by on-farm fecal, one was positive at the \geq 40 cutoff and two at the OD% \geq 20 cutoff. Of the 196 animals with at least one abattoir-positive tissue, 7.7% were positive at OD% \geq 20 and 2.6% at OD% \geq 40.

The distribution of serotypes is shown in Table 2. On the farm, only *Salmonella* Derby was isolated. At the abattoir, 17 different serotypes were isolated, including Derby. Not all isolates we sent to the National Veterinary Services Laboratories were typable due to multiple serotypes in one slant tube and contaminated or nonviable cultures. Therefore, in Table 2, the number of typable samples does not equal the number of positive samples. The proportion of different serotypes per number of positive isolates (diversity index) shows the highest diversity among CC isolates (0.18) followed by ILC (0.15) and DCC (0.14). The lowest diversity was on-farm (0), as only one serotype (Derby) was found.

The predominant serotypes ($n \ge 4$) recovered during each week of the study are shown in Table 3. The predominant serotype varied by week of the study. For example, 67% (26 of 39) of *Salmonella* Agona and 83% (20 of 24) of *Salmonella* Anatum isolates occurred during week 1. In week 2, all isolates were *Salmonella* Derby 46% (24 of 52).

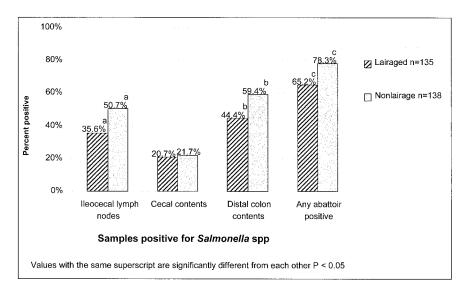


FIGURE 1. Percentage of samples from lairaged or nonlairaged market swine that were culture positive for Salmonella spp. at slaughter.

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TABLE 2. Serotypes of S. enterica isolated from various samples of market swine

Tissue/source	No. of samples positive	No. of different serotypes >1	No. of isolates typable	Diversity index ^a	Serotypes (number)
On-farm fecal	10	0	4	0.00	Derby (4)
CC	58	9	49	0.18	Agona (16), Derby (15), Typhimurium var. Copenhagen (4), Typhimurium (3), Anatum (3), Reading (2), Mbandaka (2), Braenderup (1), multiple serotypes (3)
DCC	142	15	110	0.14	Derby (29), Agona (19), Manhattan (14), Typhimurium var. Copenhagen (12), Anatum (8), Reading (5), Uganda (4), Typhimurium (3), Muenster (2), 4, 12 nonmotile (2), Infantis (1), Mbandaka (1), Montevideo (1), Senftenberg (1), Worthington (1), St. Paul (1), multiple serotypes (8)
Ileocecal lymph nodes	99	13	84	0.15	Agona (15), Derby (10), Typhimurium var. Copenhagen (6), Anatum (15), Reading (3), Uganda (4), Typhimurium (24), Manhattan (1), Muenster (1), Mbandaka (1), Montevideo (1), Senftenberg (1), Worthington (1), Braenderup (1)
Subiliac lymph nodes	1	0	0	NA	Nontypable
Ventral lymph thoracic nodes	1	0	0	NA	Nontypable
Carcass swabs	1	0	1	0.00	Typhimurium var. Copenhagen (1)

^a Diversity index = (no. of serotypes > 1)/no. of typable isolates.

Salmonella Derby was the only serotype found of the four typable on-farm isolates. One Salmonella Derby recovery occurred in study week 1; three recoveries occurred in week 2. Salmonella Derby was the only serotype isolated, at the abattoir, during every week of the study. Nearly 71% (12 of 17) of the Salmonella Typhimurium var. Copenhagen isolations occurred during week 3. The majority (83.7%, 31 of 37) of Salmonella Typhimurium isolations occurred in week 6. Of the 28 ILC cultured in week 6, 22 (78%) were positive with Salmonella Typhimurium. In week 8, there

was a cluster of *Salmonella* Manhattan isolations (76%, 13 of 17). *Salmonella* Uganda appeared only in week 9.

DISCUSSION

There was a significant difference in isolation rates from on-farm fecal samples (3.4%) and all abattoir-collected samples (71.8%) (P < 0.01). On-farm data, such as serology and fecal culture, suggest a low *Salmonella* prevalence in the pigs. Pigs that experienced about 18 h of clean lairage had lower isolation rates at slaughter than those that

TABLE 3. Number of predominate S. enterica serotypes recovered in all abattoir-collected tissues by week from market swine^a

Serotype		Week of study									
	1	2	3	4	5	6	7	8	9	10	Total
Agona	26	0	1	4	0	0	0	5	1	2	39
Anatum	20	0	0	0	0	0	0	0	3	1	24
Derby	1	24	3	1	3	4	3	4	3	6	52
Manhattan	1	0	0	0	0	3	0	13	0	0	17
Typhimurium var. Copenhagen	0	0	12	0	0	1	1	0	0	3	17
Typhimurium ^b	0	0	0	0	0	31	3	1	2	0	37
Reading	0	0	1	0	6	0	0	0	0	0	7
Uganda	0	0	0	0	0	0	0	0	4	0	4
Total typable ^c	49	24	18	12	15	39	9	36	20	22	244

^a Abattoir collected tissues = CC, DCC, ileocecal lymph nodes, carcass swabs, ventral thoracic lymph nodes, and subiliac lymph nodes. Farm fecals (not shown) resulted in one isolate of Derby in week 1 and three isolates in week 2.

^b Twenty-two of these 31 isolates in week 6 were from ileocecal lymph nodes.

^c Total typable includes other infrequently (<4) isolated serotypes not shown in this table.

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did not. In combination, these data contribute new information regarding the relative importance of sample source, transport, and lairage stress as they affect increased isolation rates from farm to abattoir.

Differences in tissue collected (CC, DCC, and ILC) and sample volume (1 versus 10 g) could explain some of the increased isolation rates between the farm and abattoir. However, if sampling alone were the cause of a rise from 3.4% (on-farm fecal) to 71.8%, then one must accept that at least 71.8% of the pigs were truly carriers on the farm. Therefore, the sensitivity of the 1-g fecal culture calculates to a very low 4.9%. However, in another study with experimentally infected known shedding pigs, we demonstrated a sensitivity of 89% for 1-g feces (11). Others have reported a relative sensitivity of 32% for 1-g feces compared to 10 or 25 g (7). Correspondingly, there are no data to suggest that serology has a sensitivity low enough to detect only two positive animals on-farm, if 196 (71.8%) truly were carriers (23). This information suggests that differences in test sensitivity are not the sole reason for a rise in prevalence, farm to abattoir.

Our study diminishes the relative importance of transport and long-term lairage as causes for increased Salmonella isolation rate. Animals were shipped under optimal conditions only 135 km in university or federal vehicles. There was minimal crowding. Until arrival at the abattoir, pigs were handled only by the farm manager or one of the investigators. If stress was a cause for increased shedding, lairaged animals should exhibit higher Salmonella prevalence, as they were commingled with new penmates, moved to new surroundings, loaded and unloaded twice, and fasted. However, Salmonella isolation rates were lower for lairaged pigs. This observation could have been due to fasting during lairage. Isaacson et al. (12) demonstrated no increase in ILC isolation rates due to transport (about 225 km) unless pigs were not fasted. Fasted pigs may have less intestinal contents from which to isolate Salmonella.

Nondifferential misclassification may have occurred due to some difficulty with individual identification of viscera. With line speeds of 1,100 pigs/h, it is possible that some (about 10%) pig identification numbers were recorded incorrectly. This misclassification was nondifferential, because it was equally likely that a viscera set would have been identified as from a control pig as from a treatment pig. This nondifferential misclassification would make it more difficult to detect a difference in prevalences among the treatment and control pigs. However, it would not bias the results in either direction. This nondifferential misclassification made it more difficult to detect a true difference between treatment and control pigs, if it existed. The fact that we observed a higher prevalence in lairaged pigs strengthens the proposition that lairage had no effect on Salmonella isolation rates.

The study was designed with sufficient sample size to detect prevalence differences of 15 percentage points on either side of 50%. Failure to detect a significant difference between treatments and controls might be attributable to insufficient sample size. However, the identification of significant difference demonstrates sufficient sample size.

Based on observations from this study, an additional explanation for increased isolation rates should be considered—acute gastrointestinal infection acquired in the abattoir holding pens. Infection was confined largely to the gastrointestinal tract and associated lymph nodes. The infection appeared to be acute, as it had not progressed beyond the first line of defensive lymph nodes. Multiple observations in this study support the acute gastrointestinal infection hypothesis. Only a short time (4 to 5 h) elapsed from the one fecal-positive lairaged pig to the 35 tissue-positive pigs at the abattoir. Based on low seropositive rates (2.7%) and only two animal isolations from systemic lymph nodes (VTL and SIL), the on-farm prevalence appears low. We assumed that infection in systemic lymph nodes might represent on-farm infection. There was a large diversity in the serotypes collected at the abattoir (n = 17) compared to the farm (n = 1), suggesting a nonfarm source for Salmonella. The clustering of different serotypes by week further supports this suggestion. There are no data to suggest that on-farm serotypes vary to the extent found in these data (4, 6, 18, 26). The weekly isolation of Salmonella Derby suggests that it was the farm's predominant serotype. We propose that pigs became viscerally infected with Salmonella while in the abattoir holding pens, resulting in higher isolation rates for gut-associated samples, such as the CC, DCC, and ILC.

The possibility of infection in long-term (>12 h) lairage has been reported (16, 20, 21). However, the possibility of short-term holding as a risk has not, to our knowledge, been substantiated with isolation of multiple Salmonella serotypes from market swine. For the acute gastrointestinal infection hypothesis to be feasible, Salmonella must spread rapidly through the gut and into visceral lymph nodes, such as the ILC. The possibility of rapid dissemination is supported by others who found Salmonella Typhimurium in the cecum of esophogostomized 6- to 8-week-old pigs, only 3 h after intranasal infection (9).

Further work is needed to determine if *Salmonella* can be detected in the gut of a market-weight pig 2 to 4 h after oral exposure. Additionally, the isolation of *Salmonella* Typhimurium, a swine-adapted pathogen, in a majority of ileocecal lymph nodes raises the possibility that this serotype infects pigs more rapidly than the other serotypes. Sampling issues remain a pertinent concern. The sensitivity of a 1-g antemortem fecal sample is not well documented. There is little correlation between carcass swab results and other abattoir-collected samples. Therefore, the value of carcass swabs for measuring food safety risk and for on-farm intervention is questionable.

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